SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 16, No. 4, pp. 762–769, 1997
Printed in the USA
0730-7268/97 $6.00 + .00

EFFECT OF FEEDING IN 30-DAY BIOACCUMULATION ASSAYS USING *HYALELLA AZTECA* IN FLUORANTHENE-DOSED SEDIMENT

GAIL A. HARKEY, SUSAN KANE DRISCOLL and PETER F. LANDRUM*
National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory,
2205 Commonwealth Boulevard, Ann Arbor, Michigan 48105-1593, USA

(*Received* 7 *February* 1996; *Accepted* 22 *August* 1996)

Abstract—Current protocols for conducting freshwater sediment bioaccumulation tests recommend that food not be added to exposures, whereas toxicity tests require food addition. To determine effects of adding food on exposure, 30-d sediment exposures were conducted with *Hyalella azteca* to sediment dosed with four fluoranthene concentrations (trace level to 897 nmol/g dry weight). Accumulation was significantly greater in fed versus nonfed animals at all dose levels after 96 h of exposure and continued to be greater after 30 d in the low dose levels. At sediment concentrations above 478 nmol/g dry weight, survival of unfed animals dropped to 34% after 30 d. After 30 d of exposure, growth and reproduction were observed in fed animals exposed to sediment concentrations 20 to 90 times the expected median lethal concentration (LC50) values for fluoranthene in sediment, according to 10-d studies reported in the literature using sediment with comparable organic carbon concentrations. Samples of sediment in exposure beakers taken from the sediment–water interface (flocculant layer) and 1 to 2 cm below the interface had large differences in fluoranthene and organic carbon concentrations. The concentration of fluoranthene was 2 to 10 times greater in the flocculant layer, the area inhabited by *H. azteca,* compared to the deeper sediment. These data raise questions concerning the interpretation of standard toxicity and bioaccumulation tests when food is routinely added.

Keywords—Sediment Bioaccumulation Fluoranthene Feeding *Hyalella azteca*

INTRODUCTION

Laboratory bioaccumulation and toxicity tests with benthic organisms are routinely used to assess effects of sediment-associated contaminants. Current protocols for freshwater sediment toxicity assays require that no food be added during the assays [1]. Toxicity studies with *Chironomus tentans* and *Hyalella azteca* indicated that additional food is required to avoid a high percentage of false positives, especially when nutrient-poor substrates are used [2]. However, guidelines for conducting 28-d bioaccumulation assays state that additional food should not be given, because less uptake of sediment-associated contaminants would presumably occur due to preferential ingestion of uncontaminated food [1,3].

In a previous toxicity study with exposure up to 30 d, mortality of *H. azteca* in sediment exposures spiked with radiolabeled fluoranthene was lower than expected, with typically greater than 85% survival. *Hyalella azteca* exposed under these conditions did not generally accumulate more than 0.75 μmol/g wet weight from sediment containing up to 876 nmol fluoranthene/g dry weight sediment [4]. This body burden is not expected to produce 50% mortality, even though median effective concentration (EC50) values were expected in the range of 11.4 to 76.1 nmol/g dry weight based on results from the literature [5,6]. In the previous study [4], organisms were fed a yeast–cerophyl–trout chow (YCT) mixture. We hypothesized that accumulation of fluoranthene in *H. azteca* may have increased if food had not been added to the exposures during the study, because selective feeding on the added uncontaminated food particles may have decreased contaminant exposure.

We tested this hypothesis by conducting bioaccumulation assays similar to those run previously to evaluate toxicity, with *H. azteca* exposed to fluoranthene-dosed sediment. Two sets of exposures (one with added food, another with no food added) at four separate dose levels were used to determine any differences in accumulation of fluoranthene over a 30-d interval. In addition, we tested the hypotheses that fed animals would be less sensitive to lethal concentrations of contaminant and that fed animals would gain significantly more weight during the bioaccumulation assays than would nonfed animals.

MATERIALS AND METHODS

Fluoranthene-dosed sediment was obtained from a previous 30-d bioaccumulation study that concluded 1 week prior to the start of the present study [4]. Sediment from the previous study was obtained from Lake Michigan at 45-m depth (43.03°N, 86.37°W) and dosed with a combination of radiolabeled [3−^{14}C]fluoranthene and non radiolabeled fluoranthene [4], and was stored at 4°C in the dark at the completion of individual exposure intervals. All analytical and bioassay procedures were performed under gold fluorescent light ($\lambda > 500$ nm) to minimize degradation of the fluoranthene and the potential for photoinduced toxicity. The sediment was assayed for contaminant concentration and purity before exposures for this study were initiated. Purity of the fluoranthene in the sediment was also assayed at the conclusion of the study. Contaminant concentration for each of the doses was quantified by liquid scintillation counting (LSC) and confirmed by extraction and quantification via gas chromatography/mass spectrometry (GC/MS).

Contaminant concentration in sediment samples was determined by placing approximately 100 mg wet weight of sediment directly into scintillation cocktail and probe sonicating

* To whom correspondence may be addressed.
Great Lakes Environmental Research Laboratory contribution 979.

the sample for 2 min [7]. Samples were corrected for quench by using the external standards ratio method after subtracting background. Concentrations of fluoranthene confirmed by GC/MS analysis [8] were spiked with deuterated standards added directly to the sediment. The samples were dessicated with precombusted sodium sulfate and extracted with 100 ml methylene chloride : acetone (1:1, v/v) by sonicating the sample for 4 min using a Tekmar high-intensity ultrasonic processor (Tekmar Corp., Solon, OH, USA; 375 W at 20% power). After settling for 24 h, the extraction solvent was decanted, filtered through glass wool, and the residue rinsed with three additional 10 ml volumes of extraction solvent. Samples were reduced to 5 ml under a stream of nitrogen using a Turbo-Vap® processor (Hopinton, MA, USA), 20 ml cyclohexane was added, and the samples reduced to 1 ml. Sample cleanup was performed on a silica gel column and the polycyclic aromatic hydrocarbon (PAH) fraction was eluted with 15% methylene chloride in hexane. The samples were spiked with deuterated internal standard and analyzed by GC/MS with selected ion monitoring [8].

Percent purity of the [3-^{14}C]fluoranthene-dosed sediment was determined by placing approximately 1 g of wet sediment in 250-ml erlenmeyer flasks, adding 75 ml acetone, and sonicating the samples for 1 h at 30°C in a 125-W sonication bath. Dichloromethane (50 ml) was added and the flasks were again sonicated 1 h at 30°C, then left to stand for 24 h at 30°C. The next day, samples were sonicated for another 1 h, filtered through glass wool, and the solvent evaporated under nitrogen to 0.5 ml using a Turbo-Vap processor. The extracts were analyzed by thin-layer chromatography (TLC) using hexane : benzene (8:2, v/v) as described previously [9].

Hyalella azteca were obtained from the National Biological Service in Columbia, Missouri, USA. Animals that passed through a 1-mm sieve but were retained on a 0.5-mm sieve were used (approximately 2–3 weeks old). Water used throughout the study was obtained from the Huron River near Ann Arbor, Michigan, USA, and was filtered (nominal pore size, 5 μm) before use. This water was chosen because the characteristics are similar to those of Lake Michigan water where Lake Michigan was the source of the sediment [4].

Four concentrations of sediment containing approximately 0.1 (trace level), 89, 478, and 897 nmol/g dry weight fluoranthene were used. These levels correspond to 0.02, 18, 97, and 181 μg/g dry weight sediment. Sediment (50 g wet weight) and overlying water (250 ml) were added to 300-ml exposure beakers 1 d before the addition of animals. Water renewal systems were constructed according to the design of Zumwalt et al. [10]. Overlying water was exchanged twice daily to minimize buildup of bacterial growth and nitrogenous wastes. Overlying water quality (dissolved oxygen [DO] and pH) was measured at the beginning, middle (day 17), and end of the study. Ten animals were added to each beaker on day 0. One milliliter of YCT in the equivalent of 2.4 mg dry weight was added to half of the beakers every day [11]. No food was added to the remainder of the beakers.

Three to five replicate exposure beakers from each of the four dose levels were sampled at the completion of 1, 2, 3, 10, 17, and 30 d. At the completion of each exposure interval, the upper 2 to 5 mm of sediment from replicate beakers of fed and unfed exposures, designated the flocculant layer, was carefully pipetted from the top of the sediment for fluoranthene and organic carbon concentrations. In addition, samples of sediment taken near the bottom of the exposure beakers (1–2 cm below the sediment–water interface) were sampled for fluoranthene and organic carbon concentration. Animals were gently removed from the sediment by sieving through a 0.5-mm sieve. The numbers of live and dead animals found were recorded. Animals from individual beakers were removed from the sediment, rinsed in distilled water, blotted dry, weighed, and placed in xylene-based scintillation cocktail (3a70b; Research Products International, Mt. Prospect, IL, USA). Samples were probe-sonicated using a Tekmar Sonicator (Tekmar Corp.) for 1 min and allowed to stand for at least 24 h prior to LSC [7].

Sediment and flocculant samples taken during the experiment were analyzed by LSC as previously described for sediment analysis. Sediment and flocculant samples were weighed and dried at 90°C to constant weight for wet to dry weight ratios. The total organic carbon content (TOC) of sediment and flocculant samples was determined by drying the sediment to constant weight, treating with 1 N HCl to remove carbonates, redrying, and assaying organic carbon on a Perkin-Elmer 2400 CHN Elemental Analyzer (Perkin-Elmer, Norwalk, CT, USA).

Differences in organism weight and contaminant accumulation between fed and unfed animals, as well as differences in TOC among the sediments were determined for each timed interval by using Student's *t* tests. Differences were considered significant at $p < 0.05$.

RESULTS

Survival and growth of *H. azteca* were significantly different between fed and unfed exposures by the completion of the 30-d study. Mean survival was greater than 90% in all exposures in all sediment concentrations through 3 d, but declined to 34% in unfed exposures in the highest dosed sediment by 30 d (Table 1). Survival remained high in exposures where food was added throughout the study at all dose levels, and reproduction was indicated after the 30-d exposure interval to yield 112 to 124% mean survival. No juvenile animals were detected in any sediment exposures after 17 d, although animal recovery was greater than 10 animals from two of the replicates. Some small amphipods were recovered from sediments after 30 d, but fewer than might be expected if reproduction was responsible for the observed increase. For example, 18 animals were recovered from a beaker after 30 d, but only 3 of the animals appeared to be juveniles. The possibility exists that small individuals not deteced at 17 d may have gained enough weight over the remainder of the study as to not be identified as juveniles after 30 d. Therefore, the number of new individuals produced over the course of the study was not reported, and survival data is reported for the total number of individuals recovered from the exposures. Weight gain of *H. azteca* was significantly different between fed and unfed animals in the 89 nmol/g dose after 3 d (Table 2). Wet weights of unfed animals from each of the sediment exposure concentrations were not different between the start and after 30 d of exposure. However, in exposures where animals were fed, animal weights were two to three times greater than those of unfed animals at all dose levels after 30 d. Growth rate coefficients taken from fed exposures ranged from 0.036 to 0.046/d when calculated as the regression of natural log of wet weight (mg) versus days of exposure (r^2 = 0.87–0.90). For the unfed animals there was no significant growth, thus the growth rates were significantly different between fed and unfed organisms at all doses. Water quality measurements were

Table 1. Comparison of survival (%) of *Hyalella azteca* in exposures where food was or was not added. All values are means of three to five replicate beakers examined after 1 to 30 d. Numbers in parentheses represent the range of percent survival[a]

Days of exposure	Treatment	Fluoranthene dose level (nmol/g dry weight)			
		Trace	89	478	897
1	Fed	93.3 (90–100)	96.7 (90–100)	100.0 (100)	103.3 (100–110)
	Unfed	93.3 (90–100)	95.0 (90–100)	93.3 (80–100)	96.7 (90–100)
2	Fed	100.0 (70–100)	100.0 (100)	93.3 (90–100)	93.3 (80–100)
	Unfed	90.0 (70–100)	85.0 (70–100)	93.3 (80–100)	100.0 (100)
3	Fed	100.0 (100)	96.7 (90–100)	96.7 (90–100)	93.3 (80–100)
	Unfed	93.3 (90–100)	93.3 (80–100)	93.3 (90–100)	100.0 (100)
10	Fed	90.0 (80–100)	92.0 (90–100)	106.0 (100–130)	84.0 (60–100)
	Unfed	88.0 (70–100)	92.5 (80–100)	78.0 (60–90)	78.0 (50–100)
17	Fed	100.0 (90–110)	100.0 (100)	93.3 (70–110)	86.7 (80–100)
	Unfed	93.3 (80–100)	95.0 (90–100)	56.7 (40–90)	63.3 (50–80)
30	Fed	116.0 (90–180)	124.0 (90–190)	116.0 (90–200)	112.5 (60–150)
	Unfed	82.0 (70–110)	77.5 (70–90)	64.0 (50–80)	34.0 (10–50)

[a] Survival greater than 100% indicates reproduction in the later exposures.

Table 2. Comparison of weight gain by *Hyalella azteca* in exposures where food was or was not added. Values are the mean of three to five groups of animals measured in milligrams wet weight ± 1 standard deviation

Days of exposure	Treatment	Fluoranthene dose level (nmol/g dry weight)			
		Trace	89	478	897
1	Fed	0.544 (0.056)	0.546 (0.039)	0.628 (0.067)	0.582 (0.086)
	Unfed	0.673 (0.194)	0.597 (0.089)	0.577 (0.404)	0.538 (0.043)
2	Fed	0.524 (0.004)	0.535 (0.080)	0.627 (0.062)	0.632 (0.037)
	Unfed	0.527 (0.065)	0.532 (0.117)	0.622 (0.128)	0.583 (0.006)
3	Fed	0.557 (0.101)	0.675* (0.040)	0.532 (0.079)	0.575 (0.051)
	Unfed	0.525 (0.076)	0.531 (0.050)	0.581 (0.135)	0.565 (0.070)
10	Fed	1.040* (0.121)	0.942* (0.079)	0.739* (0.106)	0.620 (0.041)
	Unfed	0.593 (0.086)	0.583 (0.052)	0.586 (0.054)	0.612 (0.099)
17	Fed	1.527* (0.138)	1.371* (0.037)	1.205* (0.122)	1.253* (0.376)
	Unfed	0.540 (0.134)	0.542 (0.079)	0.524 (0.051)	0.555 (0.128)
30	Fed	1.735* (0.335)	1.545* (0.194)	1.543* (0.305)	1.563* (0.290)
	Unfed	0.568 (0.054)	0.542 (0.083)	0.536 (0.092)	0.674 (0.435)

* Weight was greater in exposures where food was added in comparison to unfed exposures, using Student's *t* test at the 0.05 level of significance.

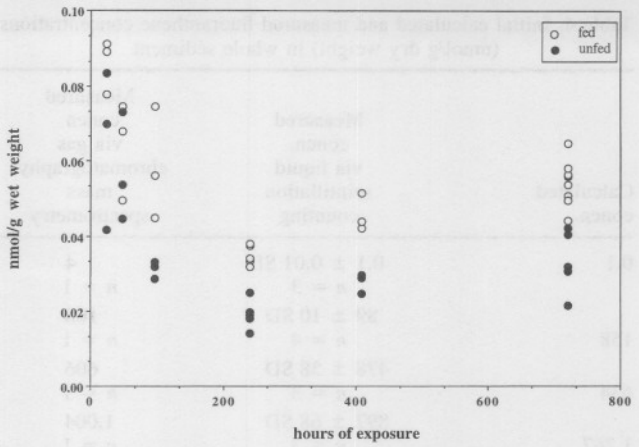

Fig. 1. Concentration of fluoranthene in *Hyalella azteca* after 1-, 2-, 3-, 10-, 17-, and 30-d exposures in sediment dosed with trace levels of the contaminant, when food was or was not added. Concentration means ± 1 SD nmol/g for fed animals are 0.086 ± 0.007 (day 1), 0.064 ± 0.013 (day 2), 0.059 ± 0.015 (day 3), 0.035 ± 0.003 (day 10), 0.046 ± 0.005 (day 17), and 0.055 ± 0.006 (day 30). Concentration means ± 1 SD nmol/g for unfed animals are 0.065 ± 0.021 (day 1), 0.057 ± 0.015 (day 2), 0.031 ± 0.002 (day 3), 0.019 ± 0.004 (day 10), 0.028 ± 0.003 (day 17), and 0.033 ± 0.008 (day 31).

not different between fed and unfed exposures. Dissolved oxygen ranged between 6.8 and 8.1 mg/L (80–95% saturation) and pH was 8.0 to 8.3 in all beakers over the course of the study.

Accumulation of fluoranthene in *H. azteca* peaked within 24 h in both fed and unfed animals in the trace and 89 nmol/g dose levels and for unfed animals in the 478 and 897 nmol/g levels (Figs. 1 to 4). Accumulation peaked by 3 d in fed animals at the two highest dose levels. After the initial peak, accumulation dropped considerably in animals exposed to all levels of fluoranthene-dosed sediment except for fed animals in the 897 nmol/g level where accumulation leveled off after 96 h (Fig. 4). For all other animals, accumulation again increased to the end of the 30-d exposures. Fluoranthene accumulation

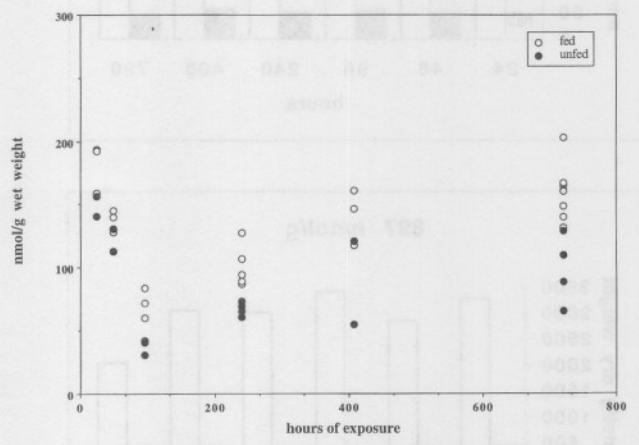

Fig. 2. Concentration of fluoranthene in *Hyalella azteca* after 1-, 2-, 3-, 10-, 17-, and 30-d exposures in sediment dosed with 89 nmol/g dry weight levels of the contaminant, when food was or was not added. Concentration means ± 1 SD nmol/g for fed animals are 181.9 ± 19.8 (day 1), 137.5 ± 8.9 (day 2), 71.7 ± 11.8 (day 3), 100.9 ± 16.6 (day 10), 141.4 ± 21.9 (day 17), and 156.6 ± 21.3 (day 30). Concentration means ± 1 SD nmol/g for unfed animals are 148.4 ± 11.2 (day 1), 121.5 ± 12.7 (day 2), 38.0 ± 6.2 (day 3), 67.1 ± 5.2 (day 10), 87.5 ± 46.6 (day 17), and 97.9 ± 27.2 (day 31).

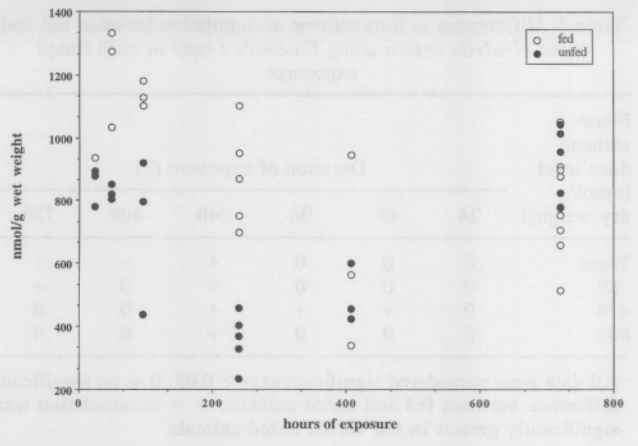

Fig. 3. Concentration of fluoranthene in *Hyalella azteca* after 1-, 2-, 3-, 10-, 17-, and 30-d exposures in sediment dosed with 478 nmol/g dry weight levels of the contaminant, when food was or was not added. Concentration means ± 1 SD nmol/g for fed animals are 898.2 ± 32.6 (day 1), 1,212.8 ± 157.7 (day 2), 1,137.1 ± 39.7 (day 3), 874.7 ± 161.0 (day 10), 617.3 ± 307.0 (day 17), and 819.0 ± 170.2 (day 30). Concentration means ± 1 SD nmol/g for unfed animals are 850.8 ± 60.8 (day 1), 835.4 ± 24.3 (day 2), 717.9 ± 251.0 (day 3), 358.6 ± 89.9 (day 10), 493.6 ± 94.6 (day 17), and 924.2 ± 115.5 (day 31).

was significantly greater in fed animals exposed to all dose levels after 96 and 240 h of exposure, and continued to be greater in animals fed at the lowest dose level (Table 3). However, no accumulation differences between fed and unfed animals were observed after 408 h at the two highest dose levels of sediment. Thus, in a typical 10-d sediment toxicity bioassay (240-h duration), *H. azteca* that were fed extraneous food would be expected to have greater exposure than unfed organisms under these exposure conditions.

Measured sediment concentrations of fluoranthene in the four levels used in the study were generally lower than the nominal calculated concentrations at the time that the sediments were originally dosed (Table 4). The sediments contained approximately 4 nmol/g of native fluoranthene (fluor-

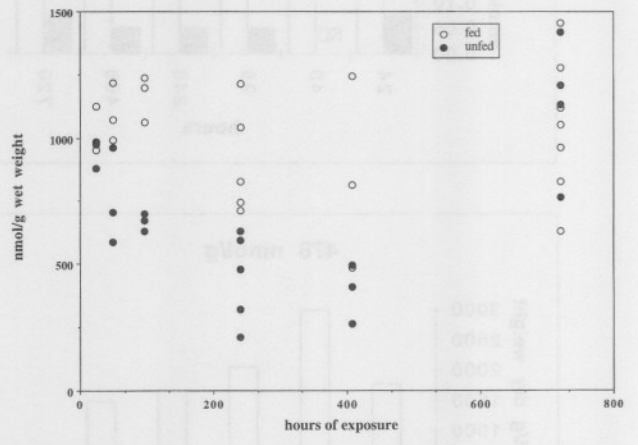

Fig. 4. Concentration of fluoranthene in *Hyalella azteca* after 1-, 2-, 3-, 10-, 17-, and 30-d exposures in sediment dosed with 897 nmol/g dry weight levels of the contaminant, when food was or was not added. Concentration means ± 1 SD nmol/g for fed animals are 1,019.4 ± 91.6 (day 1), 1,093.8 ± 113.1 (day 2), 1,164.9 ± 91.0 (day 3), 907.8 ± 214.4 (day 10), 846.0 ± 380.4 (day 17), and 1,091.6 ± 286.3 (day 30). Concentration means ± 1 SD nmol/g for unfed animals are 947.4 ± 59.1 (day 1), 751.0 ± 192.4 (day 2), 667.4 ± 33.7 (day 3), 446.5 ± 177.4 (day 10), 389.3 ± 116.7 (day 17), and 1,220.1 ± 311.3 (day 31).

Table 3. Differences in fluoranthene accumulation between fed and unfed *Hyalella azteca* using Student's *t* tests or each timed exposure[a]

Fluoranthene dose level (nmol/g dry weight)	Duration of exposure (h)					
	24	48	96	240	408	720
Trace	0	0	0	+	+	+
89	0	0	0	+	0	+
478	0	+	+	+	0	0
897	0	0	0	+	0	0

[a] All data were considered significant at $p > 0.05$. 0 = no significant difference between fed and unfed animals; + = accumulation was significantly greater in fed versus unfed animals.

Table 4. Initial calculated and measured fluoranthene concentrations (nmol/g dry weight) in whole sediment

Calculated concn.	Measured concn. via liquid scintillation counting	Measured concn. via gas chromatography/mass spectrometry
0.1	0.1 ± 0.01 SD $n = 3$	4 $n = 1$
158	89 ± 10 SD $n = 4$	108 $n = 1$
634	478 ± 58 SD $n = 3$	606 $n = 1$
1,267	897 ± 68 SD $n = 3$	1,004 $n = 1$

anthene concentration prior to sediment dosing), which influenced the total concentration at the trace level but would have had minimal influence on the other concentrations studied. Other than for the trace concentration, the GC/MS analyses confirmed the concentrations as determined by radiometric measurement. Concentration differences between the two methods at the trace level was most likely due to the fluoranthene being close to the limit of detection for the GC/MS methodology (limit of detection = 3 ng/g sediment). Sediment concentrations measured during the study that previously used these sediments were also lower than those calculated at the time of dosing due to incomplete sorption of the contaminant during the spiking process [4]. Significant differences in sediment concentrations were observed between the flocculant material (sediment taken from the upper 2–5 mm) and sediment sampled deeper in the exposure beakers (Fig. 5). Concentrations of fluoranthene in the flocculant layers were 2 to 10 times greater than in the deeper sediment. Normalizing the concentrations to organic carbon resulted in similar concentrations between the two sediment layers (Table 5). Although the deeper sediment concentrations remained nearly the same throughout the 30-d study, flocculant concentrations tended to decline from 1 or 2 d to 30 d. This was most apparent in the 89 and 478 nmol/g dose levels (Fig. 5). However, no differences in fluoranthene concentration between fed and unfed exposures

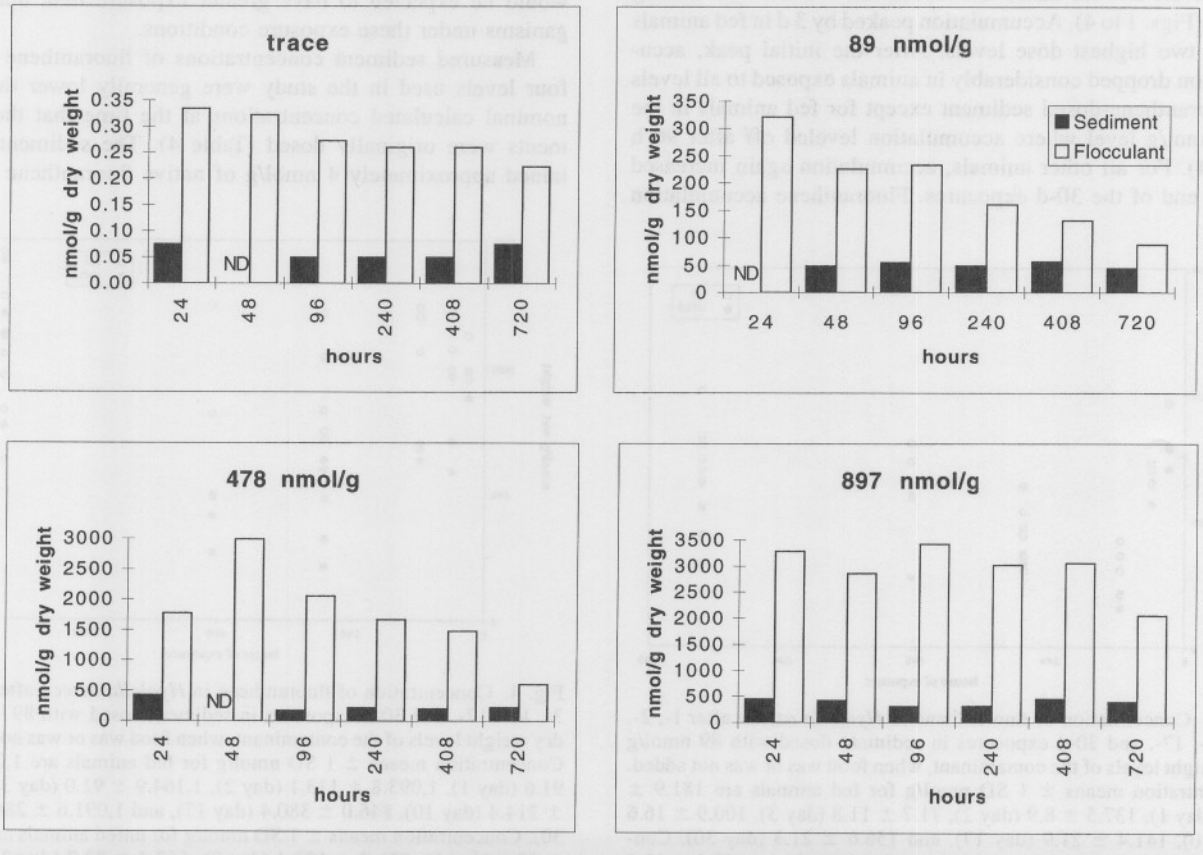

Fig. 5. Fluoranthene concentrations of the flocculant and deeper sediment sampled from exposure beakers after 1, 2, 3, 10, 17, and 30 d, for each of the four dose levels. Values are means of four replicate samples taken from beakers where food had or had not been added. No significant differences in concentration between fed and unfed beakers were observed for flocculant layers or for deeper sediments. ND = no data available.

Table 5. Organic-carbon-normalized concentration (μmol/g organic carbon) for flocculant and sediment samples taken after 24, 240, and 720 h of exposure for the four dose levels[a]

	Flocculant		Sediment	
	Unfed exposures	Fed exposures	Unfed exposures	Fed exposures
Trace dose level				
24	0.02	0.025	0.015	0.025
240	0.02	0.01	0.01	0.01
720	0.02	0.015	0.015	0.025
89 nmol/g				
24 h	—[b]	22.5	—	—
240 h	12.5	8.4	15.4	11.7
720 h	6.8	5.3	14.9	12.2
478 nmol/g				
24 h	125.2	124.3	96.8	88.8
240 h	138.8	89.6	33.9	47.3
720 h	44.8	42.7	85.9	49.8
897 nmol/g				
24 h	226.7	200.8	46	—
240 h	199	177.6	120.4	108.9
720 h	147.8	113.6	120.1	84.5

[a] Molecular weight 202.
[b] — = data not available.

were observed for either the deep sediment or the flocculant material. All fluoranthene-dosed sediments were found to contain at least 93% parent compound prior to the start of the study and greater than 90% parent compound at the conclusion of the study, suggesting that the dominant exposure was to parent compound.

Organic carbon content of the flocculant material was also significantly greater than deeper sediment (Table 6). Organic carbon in the flocculant layer averaged 1.46% after 1 d of exposure, whereas that in the deeper sediment averaged 0.45%. Although differences in organic carbon were not observed between fed and unfed exposures after 1 and 17 d of exposure, carbon content after 30 d was significantly greater in beakers that had received food than in beakers that had not received food, both for flocculant layers and deeper sediment samples (Table 6).

DISCUSSION

Methods for measuring the bioaccumulation of sediment-associated contaminants with freshwater invertebrates state that food should not be given during a test, because the addition of food may obscure the bioavailability of contaminants in sediment [1,3]. Some researchers have hypothesized that adding food during a bioaccumulation test may alter the exposure of test organisms to contaminants if the organisms preferentially feed on the uncontaminated food [12]. Evidence of this was indicated in a toxicity test when adding food to exposures weakened the response of oligochaetes to sediment-associated heavy metals [13]. Bioavailability was also shown to be altered when food was given to larvae *Chironomus riparius* exposed to a variety of sediment-associated organic contaminants [14]. However, the accumulation of the contaminants was both positively and negatively correlated with feeding, depending on the contaminant considered. The data from the present study show that fed animals exposed to contaminated sediment accumulated significantly more fluoranthene than unfed animals after 48 to 96 h of exposure and continued to accumulate more compound in the two lower dosed sediments to the end of the 30-d study. This negated our hypothesis that unfed animals would accumulate more fluoranthene than fed animals during a bioassay, and does not explain the lack of expected mortality and fluoranthene accumulation observed in a previous study that used the same feeding protocol as the present study [4]. Furthermore, in exposures where food was added, organisms gained considerable weight and reproduced, even when sediment was dosed with concentrations of fluoranthene approximately 20 to 90 times the EC50 reported for 10-d exposures with comparable levels of sediment organic carbon [4].

The elevated mortality in unfed treatments at the two highest fluoranthene concentrations may have resulted from the stress associated with starvation, because organic carbon content of the sediment did not exceed 1.6% (whole sediment contained approximately 0.5% organic carbon). Such stress placed on the unfed animals may have caused a general lowered metabolic rate so that feeding was slowed and contaminant uptake was lowered.

Mortality due to narcosis is one response mechanism thought to be responsible for the toxic effects of PAHs at concentrations ranging from 2 to 8 μmol/g wet weight for acute responses, to 0.2 to 0.8 μmol/g for chronic exposures in fish [15]. Similar values have been reported for freshwater invertebrates (e.g., *Diporeia* spp., 6–9 μmol/g for pyrene and a mixture of PAHs [16,17]). In the present study, animals accumulated up to 1.4 μmol/g wet weight after 30 d in the highest sediment concentration exposures. This body burden might be expected to result in some level of significant mortality by narcosis, although not 50%. Previous 10-d water-only exposures with *H. azteca* performed in our laboratory show that an average body burden of 5.6 μmol/g wet weight fluoranthene is associated with 50% mortality [18]. The difference between body burdens required to produce 50% mortality by fluoranthene narcosis and body burdens observed seemed sufficient to explain the low mortality in this study. The relatively low accumulation of fluoranthene by *H. azteca* in these ex-

Table 6. Mean total organic carbon (%) of flocculant layers and deeper sediment sampled from exposure beakers after 1, 17, and 30 d of exposure. Values in parentheses = ± 1 standard deviation

	1 d		17 d		30 d	
	Flocculant	Sediment	Flocculant	Sediment	Flocculant	Sediment
Beakers that received food	1.450 (0.052) $n = 8$	0.432 (0.125) $n = 4$	1.495 (0.180) $n = 8$	0.411 (0.094) $n = 8$	1.626 (0.092) $n = 8$	0.409 (0.068) $n = 8$
Beakers that did not receive food	1.471 (0.069) $n = 6$	0.462 (0.141) $n = 4$	1.484 (0.54) $n = 8$	0.419 (0.178) $n = 8$	1.269 (0.136) $n = 8$	0.324 (0.037) $n = 8$

periments may result from rapid elimination of fluoranthene by *H. azteca* ($t_{1/2}$ = 3–6 h [18]) when they come to the surface of the sediment, thereby reducing their sediment contact. Further studies will determine whether this phenomenon is specific to the particular contaminant or species studied.

Although we had hoped to be able to compare the rates of contaminant uptake for the animals exposed to the four concentrations of fluoranthene-dosed sediment, we could not accurately model the toxicokinetics due to the shape of the uptake curves obtained. Rapid uptake of fluoranthene occurred, and fluoranthene concentrations in animal tissues peaked before the first sampling point for most exposures (Figs. 1 to 4). This suggests a rapid accumulation of contaminant from interstitial water. The initial accumulation peak was followed by a rapid decline in tissue concentration, then another increase to the end of the 30-d study, in most cases. Changes in bioavailability of the contaminant or changes in behavior throughout the exposure intervals apparently caused the fluctuations in these uptake curves. Data needed for toxicokinetic models that incorporate these changes, such as growth dilution, metabolic rates, and contaminant sorption/desorption rates from food and sediment particles are not presently available, and without these parameters, an accurate estimation of contaminant uptake rates cannot be made.

Significant differences in fluoranthene accumulation between fed and unfed animals were not apparent after day 10 at the two highest sediment concentrations. These differences in accumulation cannot be explained at the present time and further tests are needed to show if this trend consistently occurs in animals exposed to fluoranthene concentrations in excess of 89 nmol/g dry weight.

The rapid initial rise then decline in accumulation has been reported for low molecular weight PAHs (<approx. 230 mol. wt. [8,19]), and has been suggested to result from rapid uptake of the dissolved concentration of contaminant in pore water. As this concentration is depleted, the initial uptake rate is slowed. Desorption from sediment particles is not rapid enough to maintain the initial pore-water concentration, and bioavailability declines. The subsequent rise in fluoranthene availability (e.g., the increase in accumulation) after 10 d in the two lowest sediment concentrations may be due to increased ingestion of the contaminant, which may be the effect of physiological changes that occurred in animals as part of the aging process. The same type of uptake curve was observed in unfed animals exposed to the two highest contaminant concentrations but was not as apparent in fed animals from these dose levels. The relatively stable concentration in fed animals from 3 to 30 d of exposure in the 897 nmol/g sediment dose level shows that steady state was apparently reached early, and bioavailability of the contaminant stayed constant throughout the study.

In the previous study where *H. azteca* were exposed to the two lower concentrations of the same sediment (trace and 89 nmol/g dry weight), tissue accumulation also peaked by 24 h [4]. However, no later increase in accumulation occurred; instead, tissue concentration continued to decline throughout the 30-d study. Furthermore, peak tissue concentrations only reached about 0.75 nmol/g wet weight in the animals. That greater than 93% of the fluoranthene dosed into sediments was present as parent compound before this study and greater than 90% was present after the study shows that these bioavailability differences were not likely due to degradation of the fluoranthene in the sediment. The difference in responses between the two studies that used the same dosed sediment can only be explained by the effects of sediment aging and potentially by the manipulation of the sediment during its use in the previous experiment. Sediment used in this study had aged for 3 months prior to the start of the assay; sediment in the previous study had only aged 2 months prior to being used. Bioavailability of PAHs and other organic contaminants has been reported to increase, decrease, or stay the same with increased sediment–contaminant contact time, and appears to be compound-specific [8,14,20,21].

Organic-carbon-normalized concentrations of flocculant and deeper sediment samples show minimal differences between the two sediment layers in the trace and 89 nmol/g dose levels (Table 5), but these values vary by approximately a factor of four between flocculant and deeper sediment after some exposure intervals at the 478 and 897 nmol/g dose levels. Changes in bioavailability of the contaminant to *H. azteca* may have resulted from these temporal changes in contaminant concentration (Fig. 5). The differences in fluoranthene concentration between the flocculant layer and deeper sediment suggest that bioavailability of contaminants may be significantly affected by the area occupied by indicator species in the exposure beakers, especially when greater concentrations of contaminant are present. *Hyalella azteca* were observed to occupy the uppermost section of sediment in the beakers. Sediment avoidance was not apparent, but animals were not found lower than about 1 cm below the sediment–water interface in beakers at all dose levels, normally indicative of *H. aztecas*'s epibenthic nature [11]. Concentration differences between the flocculant layer and deeper sediment were apparent from the first sampling point to the end of the study (Fig. 5) and were due to settling of the larger sediment particles containing lower fluoranthene concentrations. This observation suggests that sediments were not at a steady state over the study duration (Fig. 5). The gradual decrease in flocculant contaminant concentration over the course of 30 d was most likely due to the animals' depletion of contaminant in the "inhabited" layer of the beakers. Food added to the beakers, totaling 72 mg dry weight by the end of the study, did not contribute to the decrease in fluoranthene in the flocculant layer over time, as concentration of the contaminant in flocculant layers did not significantly differ between fed and unfed beakers. However, the accumulation of food over 30 d affected the organic carbon content of both flocculant and sediment layers in the beakers, causing TOC to be significantly greater in fed exposures than in unfed exposures.

Sediments used in toxicity and bioaccumulation are routinely mixed and/or composited prior to sampling for contaminant and organic carbon concentration [1]. Organic-carbon-normalized values are often used when reporting bioaccumulation or toxicity of sediment-associated organic contaminants, because normalized values are thought to reduce the variability among various sediment types and compositions. However, some of the data from this study show a significant variation in fluoranthene concentration between the flocculant layer and deeper sediment, even when organic-carbon-normalized values are considered. For example, the organic-carbon-normalized concentration of contaminant in the flocculant layer from the 478 nmol/g dose level after 10 d was 89.6 μmol/g organic carbon, whereas the deeper sediment value was 47.3 μmol/g organic carbon for fed organisms (Table 5). Such variation in describing the bioavailability of contaminants to indicator species may affect the interpretation of bio-

assay results. Sediment sampling techniques and the position that indicator species assume in exposures should be considered when conducting and reporting data derived from sediment bioassays.

Feeding organisms during the course of sediment assays presents a dilema to the researcher. Data from this study as well as previous studies suggest that feeding must be undertaken to result in sufficient endpoints for control survival and growth [22]. However, sediment enrichment (e.g., organic carbon loading) resulting from adding food during a bioassay may mask the effects of the contaminant, as suggested between the relative survival in the presence and absence of feeding. Additionally, fed organisms may have higher lipid content than unfed ones and thus more storage capacity for nonpolar compounds, removing those compounds from the site of action. Recent findings support this role of lipids in toxicity of nonpolar contaminants [23,24]. Our findings suggest that the current protocols for laboratory bioassays may be far from indicative of contaminant availability in natural sediments.

Acknowledgement—This work was funded in part through an interagency agreement, DW 1396659-01-0, between the U.S. Environmental Protection Agency and the National Oceanic and Atmospheric Administration (NOAA). Although the work was supported by the U.S. EPA and NOAA, it may not necessarily reflect the views of the agencies; no official endorsement should be inferred. Mention of trade names or commercial products does not constitute indorsement or recommendations for use. We wish to thank the National Biological Service, Columbia, Missouri, USA, for the gift of the *H. azteca*. This publication is a result of work sponsored by the Cooperative Institute of Limnology and Ecosystems Research (CILER) under cooperative agreements from the Environmental Research Laboratory (ERL), National Oceanic and Atmospheric Administration (NOAA), and the U.S. Department of Commerce under Cooperative Agreement NA90RAH00079.

REFERENCES

1. **U.S. Environmental Protection Agency.** 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-94/024. Technical Report Office of Research and Development, Duluth, MN.
2. **Ankley, G.T., D.A. Benoit, J.C. Balogh, T.B. Reynoldson, K.E. Day** and **R.A. Hoke.** 1994. Evaluation of potential confounding factors in sediment toxicity tests with three freshwater benthic invertebrates. *Environ. Toxicol. Chem.* **13**:627–635.
3. **American Society for Testing and Materials.** 1995. Standard guide for determination of the bioaccumulation of sediment-associated contaminants by benthic invertebrates. In *Annual Book of ASTM Standards, Water and Environmental Technology, Volume 11.05,* E 1688-95. Philadelphia, PA, pp. 1140–1189.
4. **Kane Driscoll, S., G.A. Harkey** and **P.F. Landrum.** 1997. Accumulation and toxicokinetics of fluoranthene in sediment bioassays with freshwater amphipods. *Environ. Toxicol. Chem.* **16**: 742–753.
5. **DeWitt, T.H., R. Swartz** and **J.O. Lamberson.** 1989. Measuring the acute toxicity of estuarine sediments. *Environ. Toxicol. Chem.* **8**:1035–1048.
6. **Suedel, B.C., J.H. Rodgers, Jr.** and **P.A. Clifford.** 1993. Bioavailability of fluoranthene in freshwater sediment toxicity tests. *Environ. Toxicol. Chem.* **12**:155–165.
7. **Lydy, M.J.** and **P.F. Landrum.** 1993. Assimilation efficiency for sediment sorbed benzo(*a*)pyrene by *Diporeia* spp. *Aquat. Toxicol.* **26**:209–224.
8. **Harkey, G.A., P.L. Van Hoof** and **P.F. Landrum.** 1995. Bioavailability of polycyclic aromatic hydrocarbons from a historically-contaminated sediment core. *Environ. Toxicol. Chem.* **14**: 1551–1560.
9. **Landrum, P.F., S.R. Nihart, B.J. Eadie** and **L.R. Herche.** 1987. Reduction in bioavailability of organic contaminants to the amphipod *Pontoporeia hoyi* by dissolved organic matter of sediment interstitial waters. *Environ. Toxicol. Chem.* **6**:11–20.
10. **Zumwalt, D.C., F.J. Dwyer, I.E. Greer** and **C.G. Ingersoll.** 1994. A water-renewal system that accurately delivers small volumes of water to exposure chambers. *Environ. Toxicol. Chem.* **13**:1311–1314.
11. **American Society for Testing and Materials.** 1995. Standard guide for conducting sediment toxicity tests with freshwater invertebrates E-1383-94. In *Annual Book of Water and Environmental Technology, ASTM Standards, Volume 11.05* Philadelphia, PA, pp. 802–834.
12. **U.S. Environmental Protection Agency.** 1989. Guidance manual: Bedded sediment bioaccumulation tests. EPA 600/x-89/302, ERLN-N111. Technical Report. Newport, OR.
13. **Wiederholm, T., A.M. Wiederholm** and **G. Milbrink.** 1987. Bulk sediment bioassays with five species of fresh-water oligochaetes. *Water Air Soil Pollut.* **36**:131–154.
14. **Harkey, G.A., P.F. Landrum** and **S.J. Klaine.** 1994. Preliminary studies on the effect of feeding during whole sediment bioassays using *Chironomus riparius* larvae. *Chemosphere* **28**:597–606.
15. **McCarty, L.S.** and **D. Mackay.** 1993. Enhancing ecotoxicological modeling and assessment: Body residues and modes of action. *Environ. Sci. Technol.* **27**:1719–1728.
16. **Landrum, P.F., B.J. Eadie** and **W.R. Faust.** 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia* spp. *Environ. Toxicol. Chem.* **10**:35–46.
17. **Landrum, P.F., W.S. Dupuis** and **J. Kukkonen.** 1994. Toxicokinetics and toxicity of sediment-associated pyrene and phenanthrene in *Diporeia* spp.: Examination of equilibrium-partitioning theory and residue-based effects for assessing hazard. *Environ. Toxicol. Chem.* **13**:1769–1780.
18. **Kane Driscoll, S., P.F. Landrum** and **E. Tigue.** 1997. Accumulation and toxicokinetics of fluoranthene in water-only exposures with freshwater amphipods. *Environ. Toxicol. Chem.* **16**: 754–761.
19. **Landrum, P.F.** 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi. Environ. Sci. Technol.* **23**:588–595.
20. **Harkey, G.A., P.F. Landrum** and **S.J. Klaine.** 1994. Comparison of whole-sediment, elutriate and pore-water exposures for use in assessing sediment-associated organic contaminants in bioassays. *Environ. Toxicol. Chem.* **13**:1315–1329.
21. **Landrum, P.F., B.J. Eadie** and **W.R. Faust.** 1992. Variation in the bioavailability of polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (spp.) with sediment aging. *Environ. Toxicol. Chem.* **11**:1197–1208.
22. **Ankley, G.T., D.A. Benoit, R.A. Hoke, E.N. Leonard, C.W. West, G.L. Phipps, V.R. Mattson** and **L.A. Anderson.** 1993. Development and evaluation of test methods for benthic invertebrates and sediments: Effects of flow rate and feeding on water quality and exposure conditions. *Arch. Environ. Contam. Toxicol.* **25**:12–19.
23. **Geyer, H.J., I. Scheunert, R. Brüggemann, M. Matthies, C.E.W. Steinberg, V. Zitko, A. Kettrup** and **W. Garrison.** 1994. The relevance of aquatic organisms' lipid content to the toxicity of lipophilic chemicals: Toxicity of lindane to different fish species. *Ecotoxicol. Environ. Saf.* **28**:53–70.
24. **van Wezel, A.P., D.A.M. de Vries, S. Kostense, D.T.H.M. Sijm** and **A. Opperhuizen.** 1995. Intraspecies variation in lethal body burdens of narcotic compounds. *Aquat. Toxicol.* **33**:325–342.